From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IB2005/000192	27.01.2005	28.01.2004

International Patent Classification (IPC) or both national classification and IPC
C12N9/96, C12N9/20

Applicant
CSIR

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☒ The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43*bis*.1 and 64.1) is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. IV Lack of unity of invention

1. ☐ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has:

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ not paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☒ all parts.

   ☐ the parts relating to claims Nos.

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	2-23,27,28
	No:	Claims	1,24-26,29
Inventive step (IS)	Yes:	Claims	NONE
	No:	Claims	1-29
Industrial applicability (IA)	Yes:	Claims	1-29
	No:	Claims	NONE

2. Citations and explanations

   see separate sheet

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (SEPARATE SHEET)

Re Item IV

The application lacks unity contradicting Rule 13 PCT. Rule 13 PCT states that for unity of invention to be present, all subject-matter should be linked by a single general inventive concept. The common concept linking the different methods claimed in the present application is the generation of enzyme particles (or "structures") comprising crosslinked enzyme molecules, wherein the enzymes are immobilized. This concept is not novel (see item V of the present communication). Since no other feature could be identified neither in the description nor in the claims that could be considered a "special" technical feature in the sense of Rule 13.2 PCT, **each method of claims 10-23 must be regarded as a separate potential invention**. However, the IPEA has elected to carry out examination on the subject-matter of all claims.

Re Item V

1. The document numbering corresponds to the order of citation in the search report.

2. Claims 1 and 24 (and accordingly all claims) contravene the requirements of Article 6 PCT since they refer to the expression **"enzyme structures"**. Said expression is **unclear**. However, for the purposes of preliminary examination it has been considered that **particles, or spheres** are referred to. Furthermore, the lipase crystals of D10, are also considered as "enzyme structures".

2.1 Claim 8 is unclear, the expression **"selectively force"** is unclear. For the purposes of preliminary examination said expression has been omitted.

3. The present application does not satisfy the criterion set forth in Article 33(2) PCT because **the subject-matter of claims 1, 24-26 and 29 is not new** in respect of prior art as defined in the regulations (Rule 64 PCT).

   D1 discloses a process for producing an enzyme (glucose oxidase) preparation, wherein an aqueous solution comprising an enzyme is emulsified with an hydrophobic phase (see claim 5, e.g. a perfluoropolyalkylether synthetic oil, see column 10, lines 20-23), and treated with a crosslinker (see claim 1, e.g. glutaraldehyde, see column 10, line 59), so that the enzyme is crosslinked (see claim 1).

The mixing of the two phases (hydrophilic or W, hydrophobic or O) generates inevitably droplets of the aqueous phase (containing the enzyme). In said droplets the enzyme molecules are contained, and obviously after crosslinking said enzymes, enzyme particles (or "enzyme structures") are formed.

The fact that enzyme molecules often contain hydrophilic and hydrophobic ends or faces is of standard knowledge in the art (see page 3 of the present application, lines 9-11). Thus, when an emulsion is formed, droplets are generated, and automatically the enzymes will be oriented in one or other direction depending on the distribution of their hydrophobic and hydrophilic sites. Furthermore, in view of the two phases and the chemical nature of the enzyme, the enzyme molecules will be located at the outside of the droplets, at the interface between the hydrophilic and hydrophobic phases, making that after removal of the aqueous phase, the crosslinked enzymes give result to particles with a hollow structure.

D1 also discloses a method of carrying out a reaction by using the enzyme preparation generated by the described method (see e.g. columns 11 and 12). Thus, D1 is prejudicial to the novelty of claims 1, 24-26, and 29 of the present application.

4. The present application does not meet the criteria of Article 33(1) PCT, because **the subject-matter of claims 1-29 does not involve an inventive step in the sense of Article 33(3) PCT.**

Water-in-oil and water-in-oil-in-water emulsions for the preparation of enzyme micro spheres for protein delivery were standard in the art (see e.g. abstract or figure 1 of D2; pages 53 and 54 of D4; or figure 4 of D5). As above stated, the emulsion of an aqueous phase containing the enzyme and a hydrophobic phase will form inevitably droplets of the aqueous phase were the enzyme is dissolved.

The use of additional factors (or protectants) to stabilize the enzyme was also standard in the art (see e.g. abstract of D1). The use of crosslinkers to stabilize enzyme aggregates (or particles or crystals, or "structures") was also very well known (see e.g. claim 1 of D1, or abstracts of D3, D8, D9, or D10). In particular, crosslinked aggregates of lipase were disclosed in D3 (see abstract, including two of the lipases mentioned in

claim 10 of the present application, these from Thermomyces lanuginosus and Rhizomucor miehei).

All the lipases referred to in claims 6 and 28 were known (see e.g Table 4 of D13, or Table 1 of D7). Different methods were known to immobilize enzymes (see e.g. Box 2 of D12, or page 649 of D13).

Different standard methods, like recovering the enzyme particles from the second liquid phase (claim 12 of the present application) or extracting the first liquid phase from the enzyme structures (claim 13 of the present application) are standard alternatives used in the prior art (see e.g. abstract or figure 1 of D2; pages 53 and 54 of D4; figure 4 of D5; or see Tables 1-8 of D6). Said features are merely straightforward possibilities from which the skilled person would select, in accordance with circumstances, without the exercise of inventive skill, in order to solve the problem posed.

In the absence of an unexpected effect, methods directed to said standard alternatives are considered as not inventive. **Thus, the subject-matter of claims 1-29 is considered not inventive.**

It is noted that the application documents do not contain any working examples for the subject-matter of claims 17-19. Thus, if the matter was not trivial for the skilled person, such matter would have to be considered not sufficiently disclosed.